Molecular mechanisms of ovarian carcinomas – Impact on progression, prognosis and therapy

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Table of contents

1. Publications of the thesis

Noske A, Denkert C, Schober H, Sers C, Zhumabayeva B, Weichert W, Dietel M, Wiechen K. Loss of Gelsolin expression in human ovarian carcinomas. Eur J Cancer 2005; 41: 461-469
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Noske A, Weichert W, Niesporek S, Röske A, Buckendahl AC, Koch I, Sehouli J, Dietel M, Denkert C. Expression of the nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 is a prognostic factor in human ovarian cancer. Cancer 2008; 112: 1733-1743
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Noske A, Kaszubiak A, Weichert W, Sers C, Niesporek S, Koch I, Schaefer B, Sehouli J, Dietel M, Lage H, Denkert C. Specific inhibition of AKT2 by RNA interference results in reduction of ovarian cancer cell proliferation: increased expression of AKT in advanced ovarian cancer. Cancer Lett 2007; 246: 190-200
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Noske A, Lindenberg JL, Darb-Esfahani S, Weichert W, Buckendahl AC, Röske A, Sehouli J, Dietel M, Denkert C. Activation of mTOR in a subgroup of ovarian carcinomas: correlation with p-eIF-4E and prognosis. Oncol Rep 2008; 20:1409-17
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Malek A, Bakhidze E, Noske A, Sers C, Aigner A, Schäfer R, Tschernitsa O. HMGA2 gene is a promising target for ovarian cancer silencing therapy. Int. J. Cancer 2008; 123: 348-356
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Denkert C, Budczies J, Darb-Esfahani S, Györffy B, Sehouli J, Könsgen D, Zeillinger R, Weichert W, Noske A, Buckendahl AC, Müller BM, Dietel M, Lage H. A prognostic gene expression index in ovarian cancer – validation across different independent data sets. J Pathol 2009; 218: 273-280
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Noske A*, Faggad A*, Wirtz R, Darb-Esfahani S, Sehouli J, Sinn B, Nielsen FC, Weichert W, Buckendahl AC, Röske A, Müller B, Dietel M, Denkert C. IMP3 Expression in Human Ovarian Cancer is Associated With Improved Survival. Int J Gyn Pathol 2009; 28:203-10 (*both authors contributed equally)

Impact factor: 2.074 (Journal Citation Reports 2009)

2. Summary

Epithelial ovarian cancer is one of the most lethal gynaecological malignancies, which is due to the diagnosis in an advanced stage and the resistance to conventional chemotherapies. Therefore, it is an urgent need to identify new diagnostic, prognostic and therapeutic molecules. In the setting of translational research, such molecular markers can be determined in tumor tissue and can be used for estimation of patient prognosis, planning of treatment strategies, and for prediction of therapy response. In this thesis, we investigated the expression of molecular markers in primary human ovarian carcinomas and compared expression data with clinical and pathological characteristics as well as patient survival. The molecules were further characterized by functional analyses in cell culture studies. Seven original publications are presented:

1.) We investigated the expression of the actin-binding protein Gelsolin in ovarian carcinomas and found a loss at protein and RNA levels. In cell culture, we demonstrated tumor growth suppressive activity of Gelsolin and that epigenetic modification might be responsible for its down-regulation in ovarian cancer [1].

2.) We analyzed the expression of the nuclear export protein CRM1. We observed a protein expression in a subset of ovarian carcinomas related to an aggressive biological behaviour. Inhibition of CRM1 with Leptomycin B resulted in suppression of cyclooxygenase-2 expression, reduction in cell proliferation, and induction of apoptosis in ovarian cancer cells [2].

3.) We investigated the expression of AKT and members of the mTOR pathway. We observed an increased AKT protein expression in ovarian carcinomas that was associated with advanced disease stage. We found AKT1 and AKT2 mRNA expression in cancer cell lines but no relevant AKT3 mRNA levels using RT-PCR. Treatment of cancer cells with the unselective PI3K inhibitor LY294002 and RNA interference to selectively inhibit the AKT isoforms resulted in reduction of cell proliferation. Our findings show that AKT, especially the AKT2 isoform might be responsible for ovarian cancer progression [3]. Since the PI3K/AKT pathway plays an important role in ovarian cancer, we consecutively analyzed downstream molecules like mTOR, 4E-BP1, and eIF4E. It has been shown that mTOR is an attractive target in anticancer therapy and as a basis for clinical trials, it is necessary to identify patients who will profit of this therapy and to determine the status of these molecular

targets in tumor tissues. Protein expression of p-mTOR was significantly associated with p-eIF-4E expression and serous subtype. For p-4E-BP1, a significant relation to poorly differentiated carcinomas was observed. In Kaplan-Meier analysis, increased expression of p-mTOR and p-eIF-4E was related to better overall survival in ovarian cancer patients. Following, we connected the *in vivo* data with cell culture studies and found a different protein expression pattern of p-AKT, p-mTOR, and p-4E-BP1 in ten ovarian cancer cell lines. We treated cancer cells with the mTOR inhibitor Rapamycin and observed an inhibition of cell proliferation and a suppression of p-mTOR and p-4E-BP1 as well as an up-regulation of p-AKT in cancer cell lines. These data support that this pathway is important in ovarian cancer progression and may serve as a therapeutic target [4].

4.) We observed an expression of HMGA2 in serous ovarian carcinomas using in situ RNA hybridization as well as at mRNA and protein levels in cancer cells using RT-PCR and western blot. Transient silencing of HMGA2 gene by siRNA inhibited proliferation of those cancer cells which over-express this gene initially. Stable silencing of highly expressed HMGA2 gene by shRNAi in cancer cells resulted in growth inhibition. The anti-tumoral effect of HMGA2 knockdown was confirmed *in vivo*, administration of a HMGA2-targeting construct resulted in suppression of subcutaneous tumor xenografts in athymic nude mice. These findings suggest that the HMGA2 gene represents a promising target for gene silencing therapy in ovarian cancer [5].

5.) We investigated the hypothesis that gene expression analysis of ovarian carcinomas may be used to identify patients with different outcomes independently of classical clinical predictors. We used a semi-supervised method for prediction of overall survival. Affymetrix gene expression analysis from 80 ovarian carcinomas (TOC cohort) was used for the development of a predictive model. A 300-gene ovarian prognostic index was generated and validated in a leave-one-out approach. The prognostic power of this index was confirmed in an independent data set of 118 ovarian carcinomas (Dukes cohort). In multivariate analysis, the ovarian prognostic index was independent of the post-operative residual tumor. We then constructed a combined score of the molecular data and residual tumor status, which was able to define patient groups with highly significant differences in survival. Our findings suggest that gene expression analysis can be used to generate prognostic signatures in ovarian cancer that can be validated in independent data sets [6].

6.) We analyzed the expression of the IGF-II mRNA-binding protein IMP3 in ovarian carcinomas. Increased IMP3 protein and mRNA expression was associated with better overall survival indicating that IMP3 might be involved in progression of ovarian cancer [7].

These findings provide insights in molecular mechanisms of ovarian cancer progression. Some of these molecules show potential prognostic impact or may function as therapeutic targets in this disease. The design of future clinical trials should incorporate biomarker testing to determine predictive markers for response to specific inhibitors.

3. Introduction

3.1. Epidemiology and conventional prognostic factors

Epithelial ovarian cancer is the fifth most common cause of cancer death and has the highest mortality rate among gynaecological malignancies in the Western world with estimated 14,600 deaths (5% of all cancer caused deaths in women) in the United States in 2009 [8]. It accounts for approximately 85% of all ovarian malignancies [9] and has an estimated incidence of 21,550 cases (3% of all cancer diseases in women) in the USA in 2009 [8]. Due to the lack of early clinical symptoms, the majority of the patients presents with an advanced disease stage. So far, there is no screening-method for early detection.

Traditional prognostic clinical and pathological factors in ovarian cancer are patient age, tumor stage (according to the FIGO classification), lymph node status, tumor grade, and histological subtype [10,11]. However, the most important prognostic factor in these patients is residual tumor after primary surgery [12,13].

In FIGO stage I, the disease is confined to the ovary and the 5-year survival rate is 90% but only 25% of all cases are detected at this early stage. FIGO stage II is characterized by pelvic extension of the carcinoma and a 5-year survival rate between 40-70%. FIGO III is the most common stage at time of diagnosis and classified when carcinoma disseminates in the upper abdomen or lymph node metastases occur (5-year survival less than 40%). In FIGO stage IV, the carcinoma is metastasized to distant sites and the 5-year survival rate is less than 20% [14].

Epithelial ovarian carcinomas show a heterogeneous morphology and can be subdivided in the main histological subtypes: serous, mucinous, endometrioid, and clear cell carcinomas [15]. Molecular studies have supported that these histological types are associated with different biological behaviour and response to chemotherapy [16].

3.2. Genetic alterations in ovarian cancer

The histological subtypes reflect different genetic alterations. For the most common serous subtype, two pathogenetic pathways were recently proposed [17]: The type I pathway is characterized by a step-wise process of frequent mutations in KRAS,

BRAF, ERBB2 and results in a low-grade serous carcinoma. Morphologic and molecular observations indicate a development from a benign serous tumor which progresses to a borderline tumor, and than to an invasive carcinoma. In contrast, the type II pathway contributes to high-grade serous carcinomas and is characterized by mutations in TP53 in 80%, high level of chromosomal instability, and absence of mutations in KRAS, BRAF, and ERBB2. Precursor lesions are very rarely detected. Ovarian surface epithelium or inclusion cysts are proposed as the origin site for some tumors [18]. Due to the rapid development of this tumor type it has been also suggested that they arise de novo [19]. Recently, a tubal origin was suggested [20] because approximately half of the high-grade serous carcinomas are associated with tubal intraepithelial carcinoma both showing identical TP53 mutations. Less is known about the genesis of the other histological types. It is assumed that mucinous, endometrioid, and clear cell carcinomas also evolve in the same manner as type I serous cancer [17]. Endometrioid and clear cell tumors have been associated with endometriosis that is regarded as the precursor of these tumors [21]. Hereditary ovarian cancer occurs in 10% and is related to germline mutations in BRCA1/BRCA2 and Mismatch-Repair-Genes. Most of the BRCA-associated carcinomas are of the high-grade serous subtype [22].

3.3. Molecular prognostic and predictive factors

Conventional clinico-pathological factors do not accurately classify ovarian cancer patients in relation to clinical outcome and benefit of adjuvant systemic treatment. Due to the rapid and unfavourable clinical course, it is an urgent need to identify molecular parameters which are useful in estimation of patient prognosis, planning of treatments, and selection of patients who will have a benefit of standard chemotherapies or may profit from novel molecular therapeutic strategies.

Despite many efforts in ovarian cancer research, reliable prognostic and predictive biomarkers are still lacking. Several gene expression profiling studies were performed but these gene expression signatures have not been adequately validated in other data sets so far and show very little overlap [23,24].

3.4. Current treatment concepts

The current standard therapy is surgical treatment followed by systemic chemotherapy. Since residual tumor after surgery is the main prognostic factor for overall survival in ovarian cancer, optimal surgical debulking remains key treatment. Adjuvant systemic therapy is based on carboplatin in combination with a taxane [25]. Only 15-20% of the patients will experience a long time remission after chemotherapy [26]. The majority of the patients show a good response on primary systemic therapy but most of them evolve resistance and will relapse from this disease. New cytotoxic drugs have entered clinical trials which are promising to overcome drug-resistance. Further, small-molecule inhibitors and monoclonal antibodies that target molecules ("targeted therapies") of angiogenesis, cell survival and cell growth are now entering clinical trials [27].

3.5. Aim of this thesis

In this study we aimed to identify molecular markers which may be helpful in the assessment of patient prognosis as well as may serve as novel therapeutic targets. Therefore, we investigated the expression of several molecules in ovarian cancer tissue and combined in vivo findings with additional cell culture studies.

4. Studies of the thesis

4. 1. Loss of Gelsolin expression in human ovarian carcinomas

Noske A, Denkert C, Schober H, Sers C, Zhumabayeva B, Weichert W, Dietel M, Wiechen K. Eur J Cancer 2005; 41: 461-469

In the first study, we investigated the expression of Gelsolin, an actin-binding protein, which is involved in the modulation of the actin cytoskeleton and regulation of cell growth and cell motility. First, we identified a differential gene expression of Gelsolin in ovarian carcinomas and normal ovarian tissues using a Cancer Profiling Array. In this cDNA array, we found decreased Gelsolin expression levels as compared to normal tissue. We further evaluated the Gelsolin expression at protein levels in 110 ovarian tissue samples and in six ovarian cancer cell lines. We observed a reduced expression of Gelsolin in borderline tumors and primary ovarian carcinomas compared with the epithelium of normal ovaries and benign tumors by immunohistochemistry. Decreased expression was associated with poorly differentiated (high-grade) carcinomas. No association with other clinico-pathological factors or patient survival was found. Low protein levels of Gelsolin were observed in four of six ovarian cancer cell lines. In addition, we investigated the growth regulatory function of Gelsolin in ovarian cancer cell lines using cDNA transfections. Re-expression of Gelsolin in OAW42 and ES-2 cells resulted in a suppression of tumor cell survival *in vitro.* To evaluate whether epigenetic modification is responsible for the decreased Gelsolin expression in ovarian cancer cells, we treated cells with inhibitors of DNA methylation (5-aza-2'deoxycytidine) and histone deacetylation (Trichostatin A). We observed a strong up-regulation of Gelsolin in OAW42 and OVCAR3 cells.

In summary, these findings demonstrate a role of Gelsolin in progression of ovarian cancer. The protein revealed growth suppressive activity in ovarian cancer cells. Down-regulation of Gelsolin might be mediated by epigenetic modification. It is known that genes affected by epigenetic events may serve as new targets in anti-cancer therapy. Thus, reconstitution of Gelsolin by inhibitors of histone deacetylase could be a promising therapeutic intervention in ovarian cancer.

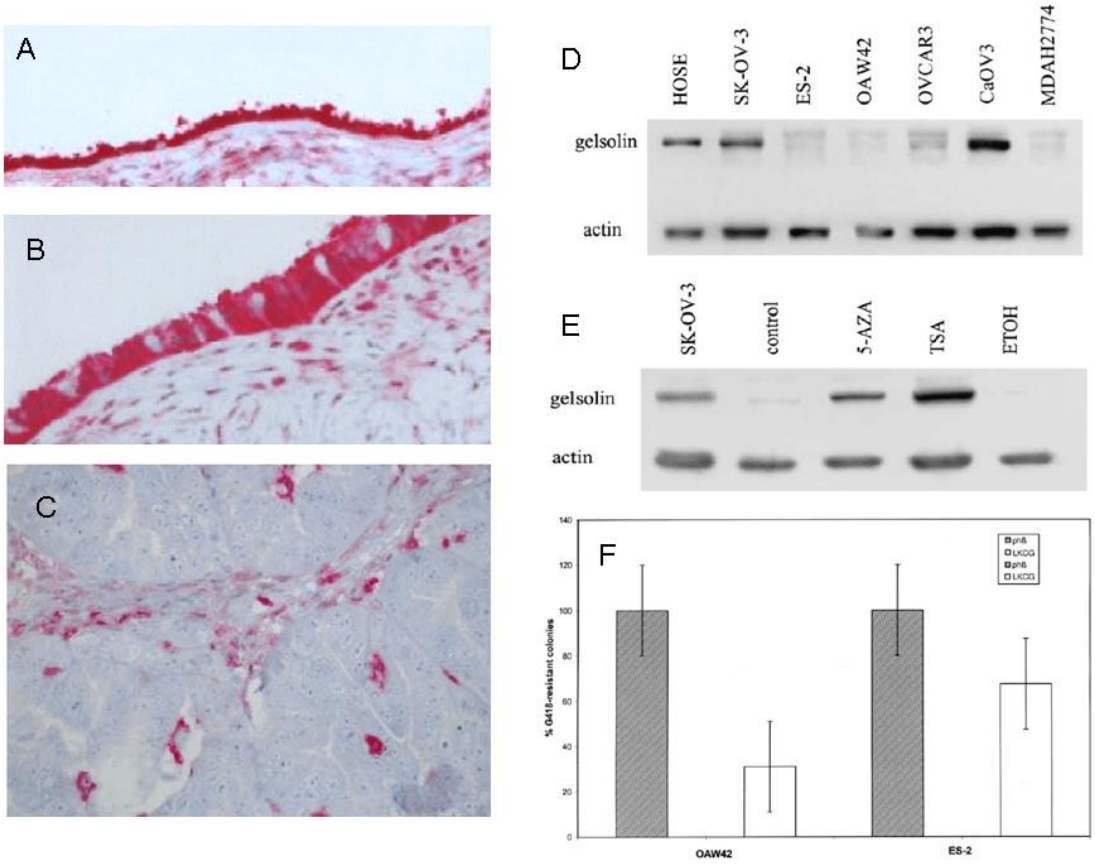

Figure 1 Immunhistochemical expression of Gelsolin in normal (A) and benign ovarian tissue (B) but loss of Gelsolin expression in a high-grade ovarian carcinoma (C). Western Blot analysis demonstrates decreased Gelsolin expression in four ovarian cancer cell lines (D). Gelsolin expression can be reconstituted in OVCAR-3 cells after treatment with inhibitors of DNA methylation and histone deacetylase (E). Colony formation assay indicates growth suppression of OAW-42 and ES-2 cells following transfection with a gelsolin expression vector (LKCG).

4. 2. Expression of the nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 is a prognostic factor in human ovarian cancer

Noske A, Weichert W, Niesporek S, Röske A, Buckendahl AC, Koch I, Sehouli J, Dietel M, Denkert C. Cancer 2008; 112: 1733-1743

In this study, we investigated the expression of CRM1 (chromosomal region maintenance/ exportin 1/ Xpo1) in primary ovarian carcinomas (n=74) and ovarian cancer cell lines (n=11). CRM1 is important in at least two areas of cellular function.

On the one hand, it serves as a nuclear-cytoplasmic transport protein and mediates an increasing number of molecules, like i. a. p53, AKT1, EGFR, and others. On the other hand, CRM1 is involved in control of chromosome structure during mitosis. It controls chromosome segregation and prevents chromosome reduplication. We observed a nuclear and cytoplasmic expression of CRM1 in 52.7% and 56.8% of the primary invasive ovarian carcinomas by immunohistochemistry but only low expression in borderline tumors (7.1%), and no expression in normal or benign ovarian tissue samples. Nuclear CRM1 expression was significantly associated with increased cyclooxygenase-2 expression. While, cytoplasmic CRM1 expression was significantly related to poorly-differentiated (high-grade) carcinomas, higher mitotic rate as well as advanced tumor stage. In univariate Kaplan-Meier analysis, nuclear CRM1 expression was significantly associated with poor overall survival for ovarian cancer patients but reached no independent prognostic significance. In western blot, we found a protein expression of CRM1 in various cancer cell lines, but reduced levels in immortalized human ovarian surface epithelial (HOSE) cells. Since COX-2 is an independent prognostic factor for overall survival in ovarian cancer patients (Denkert et al. 2002), we were interested to investigate a potential interaction between COX-2 and CRM1. We further aimed to evaluate the function of CRM1 in a cell culture model. We observed a suppression of COX-2 protein levels after incubation of OVCAR-3 cells with Leptomycin B (LMB), a specific inhibitor of CRM1. Further, CRM1 inhibition by LMB results in a significant reduction in cell proliferation using a XTT assay, decreased the percentage of cells in G1 phase, and induced apoptosis measured by using of different assays (FACS, caspase-3 activity, FITC-deoxyuridine triphosphate Apo Direct).

Our findings demonstrate that CRM1 is an interesting molecular marker for advanced and aggressive ovarian cancer. The combination of functional and *in vivo* data suggests that CRM1 may play a role in the regulation of COX-2 in ovarian cancer. Inhibition of the nuclear export of tumor relevant signalling molecules like COX-2, EGFR, p53, and others may play an important role in anticancer treatment. Novel CRM1 antagonists such as PKF050-638 or 5219668 have been recently identified and may be potential agents in targeted therapies.

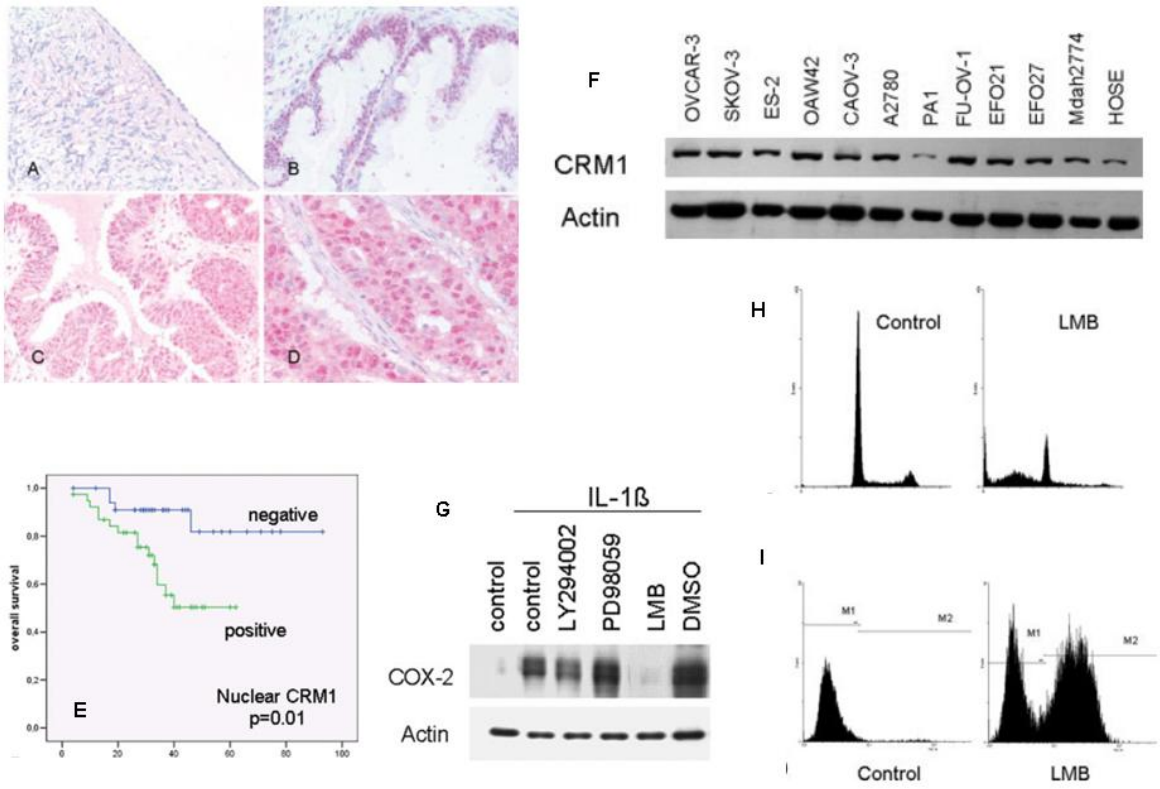

Figure 2 Immunohistochemical expression of CRM1: No immunoreaction in normal tissue (A) but intense expression in a borderline tumor (B), and invasive ovarian cancer (C, D). Kaplan-Meier analysis shows a significantly reduced overall survival in patients with CRM1 positive ovarian cancer (E). Western blot demonstrates a protein expression of CRM1 in ovarian cancer cell lines (F). COX-2 is highly inducible after treatment with IL-1β in OVCAR-3 cells (G). Incubation with Leptomycin B (LMB) results in COX-2 suppression at protein level (G). LMB-treated cells OVCAR-3 show an increased sub-G0/G1-peak and a decreased G1-phase compared with untreated cells (H). Flow cytometry indicates an increased apoptotic cell population in LMB-treated cells, represented by active caspase-3 in the M2 gate (I).

4. 3. Specific inhibition of AKT2 by RNA interference results in reduction of ovarian cancer cell proliferation: increased expression of AKT in advanced ovarian cancer

Noske A, Kaszubiak A, Weichert W, Sers C, Niesporek S, Koch I, Schaefer B, Sehouli J, Dietel M, Lage H, Denkert C. Cancer Lett 2007; 246: 190-200

In this study, we investigated the AKT expression in ovarian carcinomas and the role of AKT isoforms to ovarian cancer cell proliferation. The protein kinase AKT/PKB family has three members (AKT1, 2, and 3) but their cellular functions are not completely elucidated. AKT is known as a central signalling molecule in tumor relevant pathways and mediates cell survival. In an immunohistochemical analysis, we observed an increased AKT expression in 58% of the primary ovarian carcinomas as compared to normal ovaries. AKT expression was significantly associated with positive lymph node stage and advanced FIGO stage. In western blot analysis, total AKT expression was found in all (four) ovarian cancer cell lines, whereas the phosphorylated form was only present in OVCAR-3 and SKOV-3 cells. To evaluate the expression of the AKT isoforms, we used conventional and quantitative RT-PCR. We observed an AKT1 and AKT2 expression at the mRNA level in all cell lines, while no relevant AKT3 mRNA levels were detected. To determine the effects on cell proliferation, we used the unselective PI3K inhibitor LY294002 and RNA interference to selectively inhibit the AKT isoforms. Treatment with LY294002 and AKT2 siRNA reduced proliferation of OVCAR-3 cells. These findings show a role for AKT in ovarian cancer progression. Especially the AKT2 isoform might be responsible for ovarian cancer cell proliferation. Accordingly, AKT is an interesting target for therapeutic intervention. Meanwhile, inhibitors of AKT and PI3K (upstream of AKT) have entered clinical trials.

4. 4. Activation of mTOR in a subgroup of ovarian carcinomas: correlation with p-eIF-4E and prognosis

Noske A, Lindenberg JL, Darb-Esfahani S, Weichert W, Buckendahl AC, Röske A, Sehouli J, Dietel M, Denkert C. Oncol Rep 2008; 20:1409-17

Since the PI3K/AKT pathway plays an important role in ovarian cancer progression, we consecutively analyzed downstream molecules like mTOR, 4E-BP1, and eIF4E. The mTOR pathway is involved in cell growth and protein translation. In the last few years, it has been shown that mTOR is an attractive target in anticancer therapy. As a basis for clinical trials, it is necessary to identify patients who might have a benefit of this therapy and to determine the status of these molecular targets in tumor tissues. We found an expression of p-mTOR in 47%, p-4E-BP1 in 30.5%, and p-eIF-

4E in 55.7% of the primary ovarian carcinomas. Expression of p-mTOR was significantly associated with p-eIF-4E expression, and serous histological subtype. For p-4E-BP1, a significant relation to poorly differentiated (high-grade) carcinomas and higher mitotic rate was observed. In univariate Kaplan-Meier analysis, increased expression of p-mTOR and p-eIF-4E was related to better overall survival for patients with invasive cancer. In multivariate analysis, only postoperative residual tumor had significant independent prognostic information. We further connected the *in vivo* data with cell culture studies and found a different protein expression pattern of p-AKT, p-mTOR, and p-4E-BP1 between ten ovarian cancer cell lines using western blot. We treated cancer cells with the mTOR inhibitor Rapamycin using different incubation times and concentrations. We found an inhibition of cell proliferation after Rapamycin incubation but we did not observe any effects on cell cycle and apoptosis in this cell type. Finally, we analyzed the protein expression of mTOR signalling components after Rapamycin treatment in three cancer cell lines. We observed a suppression of p-mTOR and p-4E-BP1 as well as an up-regulation of p-AKT. These data support the importance of this signalling pathway in ovarian carcinomas and suggest that mTOR inhibition may be effective in a subset of tumors. The design of future clinical trials should incorporate biomarker testing to determine predictive markers for response to mTOR inhibitors.

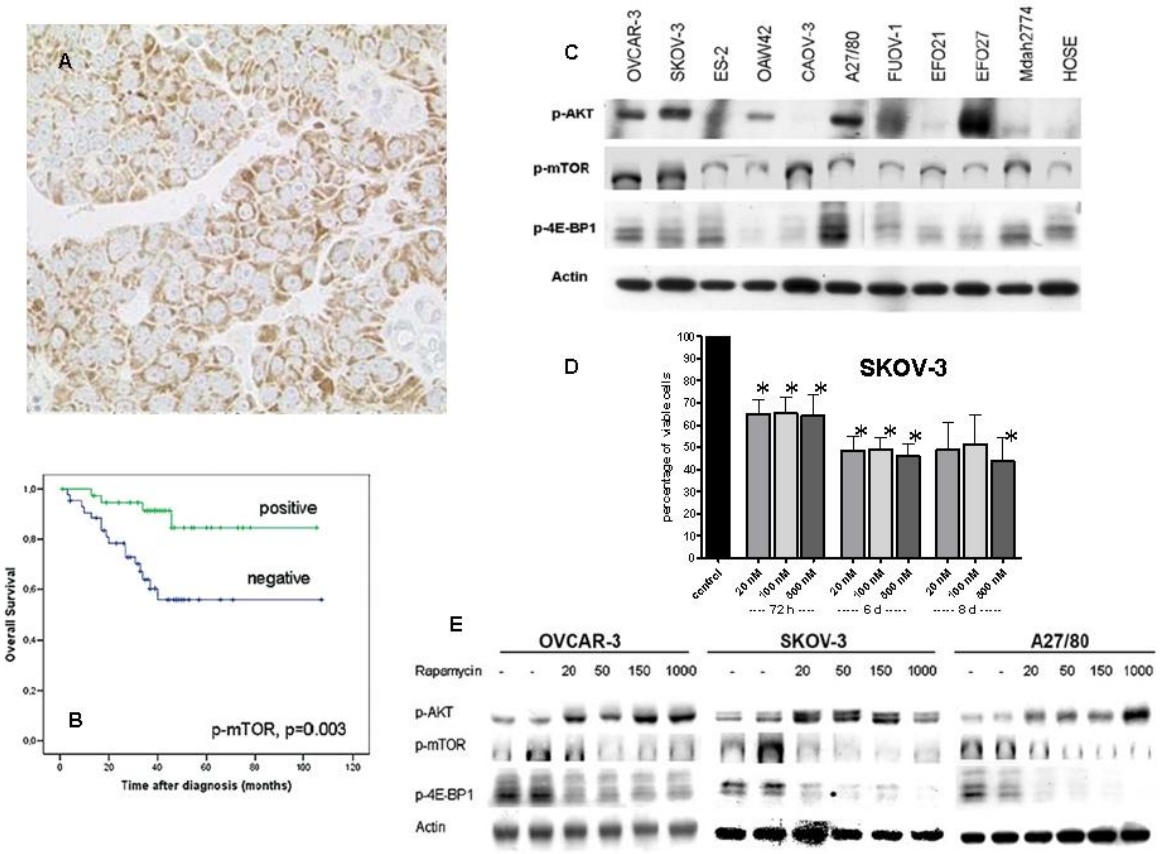

Figure 3 Immunohistochemical expression of p-mTOR in a high-grade serous ovarian carcinoma (A). Kaplan-Meier analysis displays a significant better overall survival in patients with ovarian carcinomas (B). Western blot analysis demonstrates a different expression pattern of molecules of the p-mTOR pathway (C). Rapamycin treated ovarian cancer cells show a reduced cell proliferation using an XTT assay (*$p<0.05$, two-sided t-test) (D). Rapamycin treatment reveals a over-expression of p-AKT and reduced p-mTOR and p-4E-BP1 expression (E).

4. 5. HMGA2 gene is a promising target for ovarian cancer silencing therapy
Malek A, Bakhidze E, Noske A, Sers C, Aigner A, Schäfer R, Tschernitsa O. Int. J. Cancer 2008; 123: 348-356

The goal of this study was to evaluate whether the HMGA2 gene can serve as a target for effective and safe silencing therapy of serous ovarian cancer. The HMGA2 protein belongs to the high mobility group family of nuclear nonhistone phosphoproteins. As one of the major nonhistone chromosomal proteins, HMGA

proteins are multifunctional and are involved in many cellular processes like chromatin organisation, cell-cycle control, differentiation, and cellular senescence. We investigated HMGA2 expression in 48 samples of serous ovarian carcinomas as well as five ovarian cancer cell lines. We observed an expression in 65% of the ovarian carcinomas but not in normal ovarian epithelium by using in situ RNA hybridization. Expression of HMGA2 at mRNA and protein levels was found in four cancer cell lines (OVCAR-3, SKOV-3, A27/80, OAW42) but not in CAOV-3 cells and immortalized human ovarian surface epithelial (HOSE) cells using RT-PCR and western blot. Transient silencing of HMGA2 gene by means of siRNA inhibited proliferation of those cancer cells which over-express this gene initially. Stable silencing of highly expressed HMGA2 gene by shRNAi in cancer cell lines resulted in growth inhibition because of G1 arrest and increase of apoptosis. Finally, we confirmed the antitumoral effect of HMGA2 knockdown *in vivo*. Systemic administration of a HMGA2-targeting construct resulted in suppression of subcutaneous tumor xenografts in athymic nude mice. These findings suggest that the HMGA2 gene represents a promising target for gene silencing therapy in ovarian cancer.

4. 6. A prognostic gene expression index in ovarian cancer – validation across different independent data sets

Denkert C, Budczies J, Darb-Esfahani S, Györffy B, Sehouli J, Könsgen D, Zeillinger R, Weichert W, Noske A, Buckendahl AC, Müller BM, Dietel M, Lage H. J Pathol 2009; 218: 273-280

In this study, we investigated the hypothesis that gene expression analysis of ovarian carcinomas may be used to identify patients with different outcomes independently of classical clinical predictors. We further aimed to validate these molecular markers using an independent data set. The development of such molecular markers will potentially allow the selection of those patients who will have benefit of standard therapeutic approaches or will have more profit of novel treatment strategies. We used a semi-supervised method for prediction of overall survival. At first Affymetrix gene expression analysis was performed from 80 ovarian carcinomas (TOC cohort) and used for the development of a predictive model. A 300-gene ovarian prognostic

index was generated and validated in a leave-one-out approach (Kaplan-Meier-analysis, p=0.0087). In a second validation step, the prognostic power of this index was confirmed in an independent data set of 118 ovarian carcinomas (Dukes cohort, p=0.0063). In multivariate analysis, the ovarian prognostic index was independent of the post-operative residual tumor, the main prognostic factor in this disease. We then constructed a combined score of the molecular data and residual tumor status, which was able to define patient groups with highly significant differences in survival. Our findings suggest that gene expression analysis can be used to generate prognostic signatures in ovarian cancer that can be validated in independent data sets. The integrated analysis of gene expression data and residual tumor can be used for assessment of the prognosis of platinum-taxol-treated ovarian cancer.

4. 7. IMP3 Expression in Human Ovarian Cancer is associated with improved survival

Noske A*, Faggad A*, Wirtz R, Darb-Esfahani S, Sehouli J, Sinn B, Nielsen FC, Weichert W, Buckendahl AC, Röske A, Müller B, Dietel M, Denkert C. Int J Gyn Pathol 2009; 28:203-10 (*both authors contributed equally)

In this study, we investigated the expression of IMP3 in a cohort of primary ovarian carcinomas as well as in ovarian cancer cell lines. The IGF-II mRNA-binding protein IMP3 plays an important role in embryogenesis and recent reports suggest an involvement in tumorigenesis. We observed an increased IMP3 protein expression in a subset of ovarian carcinomas that was significantly associated with better overall survival in a univariate Kaplan-Meier analysis. We confirmed these findings by a complementary method and investigated IMP3 mRNA expression in FFPE ovarian cancer specimens by real time quantitative RT-PCR. We found a highly significant correlation between protein and mRNA levels as well as a correlation of IMP3 mRNA expression with patient overall survival. We further found a protein expression in various ovarian cancer cell lines. Our results show an expression of IMP3 at protein and mRNA level in a subpopulation of ovarian cancer. These data suggest that IMP3 might be involved in progression of ovarian cancer.

5. Conclusions

In this thesis, we investigated the expression of Gelsolin, CRM1, AKT, p-mTOR, p-4E-BP1, eIF-4e, HMGA2, and IMP3 in primary epithelial ovarian carcinomas as well as in ovarian cancer cells. We observed an expression of all molecules in a subset of ovarian carcinomas. Most of them were associated with certain clinical and pathological characteristics as well as with patient survival. In functional studies, these molecules were involved in cell proliferation and showed tumor growth suppressive activity which might be interesting for anticancer treatment strategies.

In this regard, our findings confirm that the AKT/mTOR signalling pathway is hyperactivated in ovarian cancer. This pathway is frequently deregulated in ovarian cancer and plays an important role in tumor progression. Therefore, mTOR inhibition is an attractive target for antitumoral therapeutic approaches. Phase I-II trials are now ongoing with mTOR inhibitors in ovarian cancer patients [28]. Since mTOR inhibition may result in hyperactivation of AKT, combination therapies with PI3K/AKT and MAPK-inhibitors might be necessary to overcome such feedback mechanisms.
A crucial challenge for future studies will be to identify predictive biomarkers which will indicate the benefit from mTOR inhibition or other specific therapies.

To our knowledge, we investigated for the first time the expression of the export protein CRM1 in ovarian cancer specimens. It is important for transport of tumor relevant molecules like p53, COX-2 and EGFR. Therefore it is an interesting novel target for anticancer treatment. New nuclear export inhibitors (NEI) have recently shown potent antitumor effects in mouse models [29].

Taken together, the investigated molecules play a role in different cellular processes such as cell migration, cell growth and survival and alterations may contribute to the genesis and progression of ovarian carcinomas. In view of the heterogeneous biology, there is no predominant pathway that is deregulated in this tumor type. All of these defects observed within this tumor are most likely passengers and only a fraction belongs to specific operators (“drivers”). Thus, the main challenge for the future will be the identification of the “drivers” in epithelial ovarian cancer which will enable to design more effective markers and drugs for the patients [30].

Finally, more evidence became available that histological subtypes of ovarian cancer show different molecular alterations and biological behaviour. Therefore, subgroup analysis within histological subtypes should be implemented in future clinical trials.

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I am grateful to my clinical cooperation partners, particularly to Professor Dr. Jalid Sehouli, PD Dr. Martin Kruschewski, and Dr. Christiane Richter-Ehrenstein.

Finally, I would like to thank my parents.

7. References

1. Noske A, Denkert C, Schober H, et al. Loss of Gelsolin expression in human ovarian carcinomas. Eur J Cancer 2005;41:461-469
2. Noske A, Weichert W, Niesporek S, et al. Expression of the nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 is a prognostic factor in human ovarian cancer. Cancer 2008;112:1733-1743
3. Noske A, Kaszubiak A, Weichert W, et al. Specific inhibition of AKT2 by RNA interference results in reduction of ovarian cancer cell proliferation: increased expression of AKT in advanced ovarian cancer. Cancer Lett 2007;246:190-200
4. Noske A, Lindenberg JL, Darb-Esfahani S, et al. Activation of mTOR in a subgroup of ovarian carcinomas: correlation with p-eIF-4E and prognosis. Oncol Rep 2008;20:1409-1417
5. Malek A, Bakhidze E, Noske A, et al. HMGA2 gene is a promising target for ovarian cancer silencing therapy. Int J Cancer 2008;123:348-356
6. Denkert C, Budczies J, Darb-Esfahani S, et al. A prognostic gene expression index in ovarian cancer - validation across different independent data sets. J Pathol 2009;218:273-280
7. Noske A, Faggad A, Wirtz R, et al. IMP3 expression in human ovarian cancer is associated with improved survival. Int J Gynecol Pathol 2009;28:203-210
8. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225-249
9. Feeley KM, Wells M. Precursor lesions of ovarian epithelial malignancy. Histopathology 2001;38:87-95
10. Shimizu Y, Kamoi S, Amada S, Akiyama F, Silverberg SG. Toward the development of a universal grading system for ovarian epithelial carcinoma: testing of a proposed system in a series of 461 patients with uniform treatment and follow-up. Cancer 1998;82:893-901
11. Swenerton KD, Hislop TG, Spinelli J, et al. Ovarian carcinoma: a multivariate analysis of prognostic factors. Obstet Gynecol 1985;65:264-270
12. Mutch DG. Surgical management of ovarian cancer. Semin Oncol 2002;29:3-8
13. Wimberger P, Lehmann N, Kimmig R, et al. Prognostic factors for complete debulking in advanced ovarian cancer and its impact on survival. An exploratory analysis of a prospectively randomized phase III study of the

Arbeitsgemeinschaft Gynaekologische Onkologie Ovarian Cancer Study Group (AGO-OVAR). Gynecol Oncol 2007;106:69-74

14. Bast RC, Jr., Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer 2009;9:415-428
15. Cho KR, Shih Ie M. Ovarian cancer. Annu Rev Pathol 2009;4:287-313
16. Gomez-Raposo C, Mendiola M, Barriuso J, Hardisson D, Redondo A. Molecular characterization of ovarian cancer by gene-expression profiling. Gynecol Oncol 2010;118:88-92
17. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol 2004;164:1511-1518
18. Kurman RJ, McConnell TG. Precursors of endometrial and ovarian carcinoma. Virchows Arch;456:1-12
19. Bell DA, Scully RE. Early de novo ovarian carcinoma. A study of fourteen cases. Cancer 1994;73:1859-1864
20. Roh MH, Kindelberger D, Crum CP. Serous tubal intraepithelial carcinoma and the dominant ovarian mass: clues to serous tumor origin? Am J Surg Pathol 2009;33:376-383
21. Kurman RJ, Shih Ie M. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. Am J Surg Pathol 2010;34:433-443
22. Prat J, Ribe A, Gallardo A. Hereditary ovarian cancer. Hum Pathol 2005;36:861-870
23. Spentzos D, Levine DA, Ramoni MF, et al. Gene expression signature with independent prognostic significance in epithelial ovarian cancer. J Clin Oncol 2004;22:4700-4710
24. Dressman HK, Berchuck A, Chan G, et al. An integrated genomic-based approach to individualized treatment of patients with advanced-stage ovarian cancer. J Clin Oncol 2007;25:517-525
25. Harries M, Gore M. Part I: chemotherapy for epithelial ovarian cancer-treatment at first diagnosis. Lancet Oncol 2002;3:529-536
26. Kelland LR. Emerging drugs for ovarian cancer. Expert Opin Emerg Drugs 2005;10:413-424
27. Yap TA, Carden CP, Kaye SB. Beyond chemotherapy: targeted therapies in ovarian cancer. Nat Rev Cancer 2009;9:167-181

28. Trinh XB, van Dam PA, Dirix LY, Vermeulen PB, Tjalma WA. The rationale for mTOR inhibition in epithelial ovarian cancer. Expert Opin Investig Drugs 2009;18:1885-1891
29. Mutka SC, Yang WQ, Dong SD, et al. Identification of nuclear export inhibitors with potent anticancer activity in vivo. Cancer Res 2009;69:510-517
30. Darcy KM, Birrer MJ. Translational research in the Gynecologic Oncology Group: evaluation of ovarian cancer markers, profiles, and novel therapies. Gynecol Oncol;117:429-439

8. Curriculum vitae

Personal data

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Current occupation

Since 08/2009	Senior resident, Institute of Surgical Pathology, University Hospital Zurich (Prof. Dr. med. H. Moch)

Doctoral thesis

2001	Prognostic Relevance of Mismatch-Repair-Gene defects in sporadic colorectal cancer. Department of Surgery, University Hospital Benjamin Franklin, Freie Universität Berlin, Germany (Prof. Dr. med. H. J. Buhr)

Education

06/2011	Visiting Pathologist in Gynecologic Pathology, Massachusetts General Hospital, Harvard Medical School Boston (Prof. E. Oliva)

Since 07/2008	Board certified specialist in pathology
2001	licence to practice medicine
2000-2009	Resident, Institute of Pathology, University Hospital Charité Berlin, Germany (Prof. Dr. med. M. Dietel)
1996-1999	Medical study, Freie Universität Berlin, Germany
1992-1996	Medical study, Ernst-Moritz-Arndt-Universität Greifswald, Germany
1992	Matriculation, Berlin

Teaching activities

Since 2009	Courses in macroscopic and microscopic pathology for Medical students, University Hospital Zurich
2000-2009	Courses in macroscopic and microscopic pathology for Medical students, , University Hospital Charité Berlin Supervised doctoral theses

Grants

2011	Ida de Pottère-Leupold-Fonds zur Förderung der Krebsforschung (10.000 CHF)

Memberships

since 2003	American Association for Cancer Research (AACR)
since 2005	International Academy of Pathology (IAP)
since 2009	Schweizerische Gesellschaft für Pathologie (SGPath)

Review activity (Peer-review)

Biochemistry and Cell Biology, European Journal of Obstetrics and Gynaecology and Reproductive Biology, International Journal of Cancer, OncoTargets and Therapy, Virchows Archiv, BMC Cancer, Human Pathology, Breast Care, Cancer Letters

9. Publications

* Publications of the thesis

** Publications of major relevance

Original Paper

1. Kruschewski M, Noske A, Haier J, Runkel N, Anagnostopoulos I, Buhr HJ. Is reduced expression of mismatch repair genes MLH1 and MSH2 in patients with sporadic colorectal cancer related to their prognosis? Clin Exp Metastasis 2002, 19: 71-77

Impact factor: 3.91 (Journal Citation Reports 2009)

2. Denkert C, Weichert W, Winzer KJ, Müller BM, Noske A, Niesporek S, Kristiansen G, Guski H, Dietel M, Hauptmann S. Expression of the ELAV-like protein HuR is associated with higher tumor grade and increased cyclooxygenase-2 expression in human breast carcinoma. Clin Cancer Res. 2004; 15: 5880-5586

Impact factor: 6.747(Journal Citation Reports 2009)

*3. Noske A, Denkert C, Schober H, Sers C, Zhumabayeva B, Weichert W, Dietel M, Wiechen K. Loss of Gelsolin expression in human ovarian carcinomas.

Eur J Cancer 2005; 41: 461-469

Impact factor: 4.121 (Journal Citation Reports 2009)

4. Weichert W, Kristiansen G, Winzer KJ, Schmidt M, Gekeler V, Noske A, Müller BM, Niesporek S, Dietel M, Denkert C. Polo-like kinase isoforms in breast cancer: expression patterns and prognostic implications. Virchows Arch 2005; 446:442-450

Impact factor: 2.305 (Journal Citation Reports 2009)

5. Niesporek S, Denkert C, Weichert W, Köbel M, Noske A, Sehouli J, Singer JW, Dietel M, Hauptmann S. Expression of lysophosphatidic acid acyltransferase beta (LPAAT-beta) in ovarian carcinoma: correlation with tumour grading and prognosis. Br J Cancer 2005; 92; 1729-1736

Impact factor: 4.346 (Journal Citation Reports 2009)

6. Lotz K, Kellner T, Heitmann M, Nazarenko I, Noske A, Malek A, Gontarewicz A, Schäfer R, Sers C Suppression of the TIG3 tumor suppressor gene in human ovarian carcinomas is mediated via mitogen-activated kinase-dependent and -independent mechanisms. Int J Cancer 2005; 116: 894-902
Impact factor: 4.722 (Journal Citation Reports 2009)

7. Weichert W, Kristiansen G, Schmidt M, Gekeler V, Noske A, Niesporek S, Dietel M, Denkert C. Polo-like kinase 1 expression is a prognostic factor in human colon cancer. World J Gastroenterol 2005; 11: 5644-5650
Impact factor: 2.092 (Journal Citation Reports 2009)

*8. Noske A, Kaszubiak A, Weichert W, Sers C, Niesporek S, Koch I, Schaefer B, Sehouli J, Dietel M, Lage H, Denkert C. Specific inhibition of AKT2 by RNA interference results in reduction of ovarian cancer cell proliferation: increased expression of AKT in advanced ovarian cancer. Cancer Lett. 2007; 246:190-200
Impact factor: 3.741(Journal Citation Reports 2009)

9. Weichert W, Ullrich A, Schmidt M, Gekeler V, Noske A, Niesporek S, Buckendahl AC, Dietel M, Denkert C. Expression patterns of polo-like kinase 1 in human gastric cancer. Cancer Sci 2006; 97: 271-276.
Impact factor: 3.771 (Journal Citation Reports 2009)

10. Denkert C, Koch I, von Keyserlingk N, Noske A, Niesporek S, Dietel M, Weichert W. Expression of the ELAV-like protein HuR in human colon cancer: association with tumor stage and cyclooxygenase-2. Mod Pathol. 2006; 19: 1261-1269
Impact factor: 4.406 (Journal Citation Reports 2009)

11. Denkert C, Thoma A, Niesporek S, Weichert W, Koch I, Noske A, Schicktanz H, Burckhardt M, Jung K, Dietel M, Kristiansen G. Overexpression of cyclooxygenase-2 in human prostate carcinoma and prostatic intraepithelial neoplasia-association with increased expression of Polo-like kinase-1. Prostate 2007; 67: 361-369.
Impact factor: 3.081 (Journal Citation Reports 2009)

12. Niesporek S, Kristiansen G, Thoma A, Weichert W, Noske A, Buckendahl AC, Jung K, Stephan C, Dietel M, Denkert C. Expression of the ELAV-like protein HuR in human prostate carcinoma is an indicator of disease relapse and linked to COX-2 expression. Int J Oncol. 2008; 32: 341-347
Impact factor: 2.447 (Journal Citation Reports 2009)

*13. Noske A, Weichert W, Niesporek S, Röske A, Buckendahl AC, Koch I, Sehouli J, Dietel M, Denkert C. Expression of the nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 is a prognostic factor in human ovarian cancer.
Cancer 2008; 112: 1733-1743.
Impact factor: 5.418 (Journal Citation Reports 2009)

14. Niesporek S, Weichert W, Sinn B, Röske A, Noske A, Buckendahl AC, Wirtz R, Sehouli J, Koensgen D, Dietel M, Denkert C. NF-kappaB subunit p65/RelA expression in ovarian carcinoma: prognostic impact and link to COX-2 overexpression. Verh Dtsch Ges Pathol. 2007; 91: 243-249

15. Weichert W, Röske A, Niesporek S, Noske A, Buckendahl AC, Dietel M, Gekeler V, Boehm M, Beckers T, Denkert C. Class I histone deacetylase expression has independent prognostic impact in human colorectal cancer: specific role of class I histone deacetylases in vitro and in vivo. Clin Cancer Res. 2008; 14:1669-1677
Impact factor: 6.747 (Journal Citation Reports 2009)

*16. Malek A, Bakhidze E, Noske A, Sers C, Aigner A, Schäfer R, Tchernitsa O. HMGA2 gene is a promising target for ovarian cancer silencing therapy. Int J Cancer. 2008; 123: 348-56
Impact factor: 4.722 (Journal Citation Reports 2009)

17. Weichert W, Denkert C, Noske A, Darb-Esfahani S, Dietel M, Kalloger SE, Huntsman DG, Köbel M. Expression of class I histone deacetylases indicates poor prognosis in endometrioid subtypes of ovarian and endometrial carcinomas. Neoplasia 2008; 10: 1021-7
Impact factor: 5.025 (Journal Citation Reports 2009)

18. Denkert C, Budczies J, Weichert W, Wohlgemuth G, Scholz M, Kind T, Niesporek S, Noske A, Buckendahl A, Dietel M, Fiehn O. Metabolite profiling of human colon carcinoma--deregulation of TCA cycle and amino acid turnover. Mol Cancer 2008; 7: 72
Impact factor: 4.16 (Journal Citation Reports 2009)

*19. Noske A, Lindenberg JL, Darb-Esfahani S, Weichert W, Buckendahl AC, Röske A, Sehouli J, Dietel M, Denkert C. Activation of mTOR in a subgroup of ovarian carcinomas: correlation with p-eIF-4E and prognosis. Oncol Rep. 2008; 20: 1409-17
Impact factor: 1.588 (Journal Citation Reports 2009)

20. Richter-Ehrenstein C, Mueller S, Noske A, Schneider A. Diagnostic Accuracy and Prognostic Value of Core Biopsy in the Management of Breast Cancer: A Series of 542 Patients. Int J Surg Pathol. 2009; 17: 323-6
Impact factor: 0.912 (Journal Citation Reports 2009)

21. Darb-Esfahani S, Faggad A, Noske A, Weichert W, Buckendahl AC, Müller B, Budczies J, Röske A, Dietel M, Denkert C. Phospho-mTOR and phospho-4EBP1 in endometrial adenocarcinoma: association with stage and grade in vivo and link with response to rapamycin treatment in vitro. J Cancer Res Clin Oncol. 2009; 135: 933-41
Impact factor: 2.261 (Journal Citation Reports 2009)

22. Faggad A, Darb-Esfahani S, Wirtz R, Sinn B, Sehouli J, Könsgen D, Lage H, Weichert W, Noske A, Budczies J, Müller BM, Buckendahl AC, Röske A, Eldin Elwali N, Dietel M, Denkert C. Topoisomerase IIalpha mRNA and protein expression in ovarian carcinoma: correlation with clinicopathological factors and prognosis. Mod Pathol. 2009; 22: 579-588
Impact factor: 4.406 (Journal Citation Reports 2009)

*23. Denkert C, Budczies J, Darb-Esfahani S, Györffy B, Sehouli J, Könsgen D, Zeillinger R, Weichert W, Noske A, Buckendahl AC, Müller BM, Dietel M, Lage H. A

prognostic gene expression index in ovarian cancer - validation across different independent data sets. J Pathol. 2009; 218:273-280
Impact factor: 6.466 (Journal Citation Reports 2009)

24. Kleine-Tebbe A, Noske A. Benigne und präinvasive Läsionen der Brust. Gynäkol Geburtsmed Gynäkol Endokrinol 2009; 5: 264-276

25. Faggad A, Darb-Esfahani S, Wirtz R, Sinn B, Sehouli J, Könsgen D, Lage H, Noske A, Weichert W, Buckendahl AC, Budczies J, Müller BM, Elwali NE, Dietel M, Denkert C. Expression of multidrug resistance-associated protein 1 in invasive ovarian carcinoma: implication for prognosis. Histopathology 2009; 54: 657-666
Impact factor: 3.855 (Journal Citation Reports 2009)

*26. Noske A, Faggad A, Wirtz R, Darb-Esfahani S, Sehouli J, Sinn B, Nielsen FC, Weichert W, Buckendahl AC, Röske A, Müller B, Dietel M, Denkert C. IMP3 Expression in Human Ovarian Cancer is Associated With Improved Survival. Int J Gyn Pathol 2009; 28:203-10
Impact factor: 2.074 (Journal Citation Reports 2009)

27. Noske A, Lipke S, Budczies J, Müller K, Loddenkemper C, Buhr HJ, Kruschewski M. Combination of p53 expression and p21 loss has an independent prognostic impact on sporadic colorectal cancer. Oncol Rep. 2009; 22: 3-9
Impact factor: 1.588 (Journal Citation Reports 2009)

**28. Darb-Esfahani S, Wirtz R, Sinn B, Budczies J, Noske A, Weichert W, Faggad A, Scharff S, Sehouli J, Oskay-Oezcelik G, Zamagni C, De Iaco P, Martoni A, Dietel M, Denkert C. Estrogen receptor 1 mRNA is a prognostic factor in ovarian carcinoma: determination by kinetic PCR in formalin-fixed paraffin-embedded tissue. Endocr Relat Cancer 2009; 16: 1229-39
Impact factor: 5.683 (Journal Citation Reports 2009)

29. Darb-Esfahani S, Loibl S, Müller BM, Roller M, Denkert C, Komor M, Schlüns K, Blohmer JU, Budczies J, Gerber B, Noske A, du Bois A, Weichert W, Jackisch C, Dietel M, Richter K, Kaufmann M, von Minckwitz G. Identification of biology-based breast cancer types with distinct predictive and prognostic features: role of steroid

hormone and HER2 receptor expression in patients treated with neoadjuvant anthracycline/taxane-based chemotherapy. Breast Cancer Research 2009; 11: R69
Impact factor: 5.33 (Journal Citation Reports 2009)

30. Sinn BV, Darb-Esfahani S, Wirtz RM, Faggad A, Weichert W, Buckendahl AC, Noske A, Müller BM, Budczies J, Sehouli J, Braicu EI, Dietel M, Denkert C. Vascular endothelial growth factor C mRNA expression is a prognostic factor in epithelial ovarian cancer as detected by kinetic RT-PCR in formalin-fixed paraffin-embedded tissue. Virchows Arch 2009; 455: 461-467
Impact factor: 2.305 (Journal Citation Reports 2009)

31. Lehmann A, Denkert C, Budczies J, Buckendahl AC, Darb-Esfahani S, Noske A, Müller BM, Bahra M, Neuhaus P, Dietel M, Kristiansen G, Weichert W. High class I HDAC activity and expression are associated with RelA/p65 activation in pancreatic cancer in vitro and in vivo. BMC Cancer 2009; 9: 395
Impact factor: 3.15 (Journal Citation Reports 2009)

**32. Denkert C, Loibl S, Noske A, Roller M, Müller BM, Komor M, Budczies J, Darb-Esfahani S, Kronenwett R, Hanusch C, von Törne C, Weichert W, Engels K, Solbach C, Schrader I, Dietel M, von Minckwitz G. Tumor-associated Lymphocytes as an Independent Predictor of Response to Neo-adjuvant Chemotherapy in Breast Cancer. JCO 2010; 28:105-113
Impact factor: 17.793 (Journal Citation Reports 2009)

33. Noske A, Pahl S, Fallenberg E, Richter-Ehrenstein C, Buckendahl A, Weichert W, Schneider A, Dietel M, Denkert C. Flat epithelial atypia is a common subtype of B3 breast lesions and associated with non-invasive cancer but not with invasive cancer in final excision histology. Hum Pathol 2010; 41: 522-7
Impact factor: 2.961 (Journal Citation Reports 2009)

34. Darb-Esfahani S, Sinn BV, Weichert W, Budczies J, Lehmann A, Noske A, Buckendahl AC; Müller BM, Sehouli J, Koensgen D, Györffy B, Dietel M, Denkert C. Expression of classical NF-kappaB pathway effectors in human ovarian carcinoma. Histopathology 2010; 56: 727-39

Impact factor: 3.855 (Journal Citation Reports 2009)

35. Kasajima A, Sers C, Sasano H, Jöhrens K, Stenzinger A, Noske A, Buckendahl AC, Darb-Esfahani S, Müller BM, Budczies J, Lehman A, Dietel M, Denkert C, Weichert W. Down-regulation of the antigen processing machinery is linked to a loss of inflammatory response in colorectal cancer. Hum Pathol 2010; 41: 1758-69
Impact factor: 2.961 (Journal Citation Reports 2009)

36. Richter-Ehrenstein C, Arndt J, Buckendahl AC, Eucker J, Weichert W, Kasajima A, Schneider A, Noske A. Solid neuroendocrine carcinomas of the breast: metastases or primary tumors? Breast Cancer Research and Treatment 2010; 124: 413-7
Impact factor: 4.696 (Journal Citation Reports 2009)

37. Kasajima A, Pavel M, Darb-Esfahani S, Noske A, Stenzinger A, Sasano H, Dietel M, Denkert C, Röcken C, Wiedenmann B, Weichert W. mTOR expression and activity patterns in gastroenteropancreatic neuroendocrine tumours. Endocr Relat Cancer 2011; 18:181-92
Impact factor: 4.282 (Journal Citation Reports 2009)

38. Fritsche-Guenther R, Noske A, Ungethüm U, Kuban RJ, Schlag PM, Tunn PU, Karle J, Krenn V, Dietel M, Sers C. De novo expression of EphA2 in osteosarcoma modulates activation of the mitogenic signalling pathway. Histopathology 2010; 57:836-50
Impact factor: 3.855 (Journal Citation Reports 2009)

39. Fritsche-Guenther R, Gruetzkau A, Noske A, Melcher I, Schaser KD, Schlag PM, Kasper HU, Krenn V, Sers C. Therapeutic potential of CAMPATH-1H in skeletal tumours. Histopathology 2010; 57: 851-61
Impact factor: 3.855 (Journal Citation Reports 2009)

**40. Noske A, Loibl S, Darb-Esfahani S, Roller M, Kronenwett R, Müller BM, Steffen J, von Toerne C, Wirtz R, Baumann I, Hoffmann G, Heinrich G, Grasshoff ST, Ulmer HU, Denkert C, von Minckwitz G. Comparison of different approaches for

assessment of HER2 expression on protein and mRNA level - prediction of chemotherapy response in the neoadjuvant GeparTrio trial (NCT00544765). Breast Cancer Res Treat 2011; 126:109-17
Impact factor: 4.696 (Journal Citation Reports 2009)

41. Budczies J, Weichert W, Noske A, Müller BM, Weller C, Wittenberger T, Hofmann HP, Dietel M, Denkert C, Gekeler V. Genome-wide gene expression profiling of formalin-fixed paraffin-embedded breast cancer core biopsies using microarrays. J Histochem & Cytochem 2011; 59: 146-57
Impact factor: 2.37 (Journal Citation Reports 2009)

42. Buckendahl AC, Budczies J, Fiehn O, Darb-Esfahani S, Kind T, Noske A, Weichert W, Sehouli J, Braicu E, Dietel M, Denkert C. Prognostic impact of AMP-activated protein kinase expression in ovarian carcinoma: correlation of protein expression and GC/TOF-MS-based metabolomics. Oncol Rep 2011; 25: 1005-12
Impact factor: 1.588 (Journal Citation Reports 2009)

43. Noske A, Zimmermann AK, Caduff R, Varga Z, Fink D, Moch H, Kristiansen G. Alpha-methylacyl-CoA racemase (AMACR) expression in epithelial ovarian cancer. Virchows Arch 2011; 459: 91-97
Impact factor: 2.305 (Journal Citation Reports 2009)

44. Varga Z, Tubbs RR, Wang Z, Sun Y, Noske A, Kradolfer D, Bosshard G, Jochum W, Moch H, Ohlschegel C. Co-amplification of the HER2 gene and chromosome 17 centromere: a potential diagnostic pitfall in HER2 testing in breast cancer. Breast Cancer Research and Treatment 2011; 124: 413-7
Impact factor: 4.696 (Journal Citation Reports 2009)

45. Noske A, Schwabe M, Weichert W, Darb-Esfahani S, Buckendahl AC, Sehouli J, Braicu EI, Budczies J, Dietel M, Denkert C. An intracellular targeted antibody detects EGFR as an independent prognostic factor in ovarian carcinomas. BMC Cancer 2011; 11: 294
Impact factor: 3.15 (Journal Citation Reports 2009)

Case reports

1. Noske A, Pahl S. Combined adenosquamous and large-cell neuroendocrine carcinoma of the gallbladder. Virchows Arch 2006; 449:135-6.

2. Glanemann M, Morgott F, Noske A, Spinelli A, Neuhaus P. Malignes Melanom am anorektalen Übergang. Chir Gastroenterol 2006, 22: 278-282

3. Noske A, Schwabe M, Pahl S, Fallenberg E, Richter-Ehrenstein C, Dietel M, Kristiansen G. Report of a metaplastic carcinoma of the breast with multi-directional differentiation: an adenoid cystic carcinoma, a spindle cell carcinoma and melanoma. Virchows Arch. 2008; 452: 575-579

4. Pietzner K, Noske A, Cho CH, Kiecker F, Sehouli J. Amelanotic metastasis of melanoma mimicking ovarian cancer: a case report and review of the literature. Anticancer Res. 2008; 28: 563-566

5. Fritzsche F, Bode PK, Stinn B, Huber GF, Noske A. Sialolipoma of the parotid gland. Pathologe 2009; 30: 478-80

10. Abbreviations

AKT	protein kinase B (PKB/AKT)
BRCA	Breast cancer gene 1 and 2
CRM1	chromosomal region maintenance/ exportin 1
COX-2	cyclooxygenase 2
4E-BP1	eukaryotic translation inhibition factor 4E binding protein
EGFR	epidermal growth factor receptor
eIF-4E	eukaryotic initiation factor 4E
ErbB2	human epidermal growth factor receptor 2
FACS	fluorescence activated cell sorting
FIGO	Fédération Internationale de Gynécologie et d'Obstétrique
HMGA2	high mobility group A2 gene
IMP3	insulin-like growth factor-II (IGF-II) mRNA binding protein
KRAS	kirsten rat sarcoma
mTOR	mammalian target of Rapamycin
LMB	Leptomycin 1
PI3K	phosphatidylinositol-3-kinase
TOC	Tumor bank ovarian cancer network
XTT	colorimetric assay based on sodium 3´-[1-(phenylaminocarbonyl)- 3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate